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Survey, pathogenicity, cultural and morphological characterisation of *Alternaria* isolates associated with *Alternaria* leaf spot of cabbage

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Abstract
Cabbage is a profitable vegetable crop in Kerala grown for its compact head formed by the leaves. Among fungal diseases, *Alternaria* leaf spot caused by *A. brassicicola* is a noteworthy concern as it effects the crop all over the world. Hence the present study was conducted to determine the incidence of disease and detail studies of cultural and morphological characters of the pathogen. Survey was taken up in three districts of Kerala viz., Trivandrum, Kollam, Idukki and the maximum per cent disease index (57.14%) was recorded in Muthuvankudy regions of Idukki district. Seven isolates recovered from the diseased samples were isolated, purified and pathogenicity was proved. Cultural and Morphological characters of different isolates of *Alternaria* spp. obtained during the survey were studied and showed that the mycelia were septate and conidia were produced in chains from the conidiophores. Conidia are beakless and consist of both longitudinal and transverse septa.

Keywords: Cabbage, *Alternaria* leaf spot, disease incidence, cultural characters, morphological characters

Introduction
Cabbage (*Brassica oleracea* var. *capitata*) is an economically important vegetable crop which forms a compact head with leaves and grown as biennial for the production of seeds. It is an rich source of vitamin C and moderate source of vitamin K, sulphur and an amino acid glutamine. Cabbage is mainly used to treat colon cancers and its juice helps in treating ulcers and intestinal problems. The total production of cabbage in the world is 73.6 million tonnes. China is the largest producer of cabbage with 33.8 million tonnes followed by India with 8.75 million tonnes and Russia with 3.4 million tonnes. Cabbage is affected by many diseases. Among them, *Alternaria* leaf spot caused by *Alternaria brassicicola* is the most common disease causing considerable yield losses every year.

Michereff *et al.* (2012) [9] administrated a study and estimated the intensity of *A. brassicicola* and *A. brassicae* in 28 fields of crucifers in Pernambuco state of Brazil in two seasons. They reported that the distribution of *A. brassicicola* was severe than *A. brassicae* and the percentage incidence of *A. brassicicola* was 99.8% and 95.1% in cabbage and broccoli crops respectively. Rahimloo and Ghosta (2015) [12] collected thirty eight isolates of the fungus and found significant variation in cultural and morphological characters among these isolates. Studies were, therefore carried out to determine the incidence of disease, cultural and morphological characters of isolates collected from cabbage cultivated regions of Kerala.

Materials and methods
Survey
Cabbage leaves infected with *Alternaria* leaf spot were collected during the survey from three districts of Kerala viz., Trivandrum, Kollam and Idukki during 2016-2017. The data on the severity of *Alternaria* leaf spot of cabbage was recorded by using 0-5 scale score chart given by Sangeetha and Siddaramaiah, 2007 [16]. Score 0- No infection, Score I- <5% infection, Score II- 5-10% infection, Score III- 10-25% infection, Score IV- 25-50% infection, Score V- >50% infection. The Percent disease index (PDI) was determined by using the following formula (McKinney, 1923) [7]:

$$\text{Percent Disease index} = \frac{\text{Sum of grades of each leaf}}{\text{No. of leaves assessed} \times \text{Maximum grade used}} \times 100$$

Symptomatology

Symptoms of *Alternaria* leaf spot caused by *Alternaria* spp. were studied during the survey by observing the naturally infected cabbage leaves.

Isolation, Purification and Pathogenicity testing

The cabbage leaves infected with *Alternaria* spp. were collected during the survey and isolation was done by cutting small pieces of diseased portion of leaf along with healthy portion and immersed in mercuric chloride solution (0.1%) for 30 seconds. Then these leaf bits were washed thrice in sterile water and placed aseptically on the sterile petriplate which was poured with the sterilized potato dextrose agar (PDA) medium (Rahimloo and Ghosta, 2015) [12]. These plates were incubated at room temperature (27±4 ^{0}C) for proper development of the pathogen. The culture was purified by subculturing mycelium on to the PDA slants and also by single spore isolation technique (Dhingra and Sinclair, 1985) [3]. The same procedure was repeated for all the isolates collected from various locations of Kerala.

The pathogenicity was tested for all the isolates of the pathogen by make use of Koch's postulates. Cabbage leaves were artificially inoculated by following pin prick method. Before inoculation the leaves were washed with sterile water and then gentle pricking was done. Thereafter five mm culture bits were cut from seven day old culture of pathogen using sterilized cork borer and placed on wounded portion of leaves using sterilized needle. Moist cotton was placed over the mycelial bit and the control was maintained by placing culture media bits without culture of pathogen on the leaf blades after pin pricks. Inoculated plants were labelled and kept under humid conditions to maintain proper moisture for disease development. Observations were noted regarding the lesion size (cm) to identify the virulent isolate.

Cultural characters of *Alternaria* leaf spot isolates

To study the colony characters, PDA medium was prepared and sterilized in an autoclave. Then 15 ml of sterilized PDA medium was poured in sterilized petriplates under aseptic conditions. Seven days old culture of the pathogen isolates were used for this experiment. From these plates, five mm mycelial bits were cut with the help of sterilized cork borer and placed at the centre of petriplate over the media. The same procedure was repeated for all the isolates of pathogen and these plates were incubated at room temperature (27±4 ^{0}C). Cultural characters of the pathogen viz. colony colour, growth pattern, growth rate and days taken by the pathogen to cover entire petriplate were observed and recorded for all the isolates to identify the pathogen.

Morphological characters of *Alternaria* leaf spot isolates

The morphological characters of the pathogen like mycelium, conidiophore, conidia and spore development were studied by slide culture technique (Riddle, 1950) [14]. For this, blotting paper was taken and cut circularly and placed in the petridish. Then two glass rods were placed over the blotting paper. A clean microscopic slide and few coverslips were placed in petridish along with glass rods and this petridish was sterilized. Simultaneously, plain agar medium was prepared, sterilized and poured in sterilized petriplates under aseptic conditions in laminar airflow chamber.

A small block of plain agar was cut with the help of sterile scalpel blade and placed on the microscopic slide culture unit. By using inoculation needle a small portion of pathogen inoculum were taken and placed on the four sides of a block. A coverslip was placed on the agar block and gently pressed for adhesion. Thereafter sterile distilled water was added in drops over the blotting paper in order to moisten it. Then these plates were covered with lid and incubated at room temperature (27±4 ^{0}C). After one or two days the coverslip were taken and placed on another microscopic slide by adding lactophenol cotton blue stain and observed the morphological characters of the pathogen. Various attributes like mycelial width, length, breadth, colour, shape and septation of conidia, conidiophore and conidial ontogeny were observed under microscope.

Results and discussion

Survey

Survey was conducted in three cabbage cultivation tracts of Kerala viz., Trivandrum, Kollam, Idukki during 2016-17 to examine the symptomatology of the disease and to assess the Per cent Disease Index (PDI) of *Alternaria* leaf spot of cabbage. Disease incidence was observed at four locations in Idukki district (Devikulam, Selliampara, Muthuvankudy and Chithirapuram), two locations in Trivandrum (Vellayani, Kalliyoor) and in Kottarakkara from Kollam district. Seven isolates were obtained from the above mentioned locations during the survey which had been serially numbered from C1 to C7. The location, period of collection and the PDI of all these isolates were recorded and given in Table 1.

Rop *et al*. (2009) [15] conducted a survey in thirteen districts of Kenya and calculated the incidence of dark leaf spot in cabbage and kale crops. They reported that 53.8% of the cabbage and 48.6% of the kale fields were infected with the leaf spot disease. Peruch *et al.* (2006) [11] conducted a survey and reported that the PDI of *A. brassicicola* in cauliflower was 100 per cent in Brazil states. Reis and Boiteux (2010) [13] also reported that the frequency of incidence of *A. brassicicola* was high in cabbage and cauliflower where *A. brassicae* incidence was high in turnip (*B. rapa* group) in Brazilian regions. During survey, 135 isolates were collected and identified under *in vitro* conditions. Out of 8 species of *Alternaria* reported from all the isolates, the percentage incidence was high with *A. tenuissima* (39.25%) and *A. brassicicola* (28.14%) thereby caused severe economic losses to the crop (Rahimloo and Ghosta, 2015) [12].

Table 1: Isolates of *Alternaria* spp. collected from seven locations

Sl. No.	Isolate	Location	Period of collection	PDI*	Temp (^{0}C)		RH (%)	
					Min	Max	Min	Max
1	C 1	Kottarakkara, Kollam	January, 2017	19.9 (26.54)^e	23	32	37	93
2	C 2	Vellayani, Trivandrum	December, 2016	17.49 (24.7)^f	24	32	40	100
3	C 3	Kalliyoor, Trivandrum	December, 2016	29.15 (32.66)^d	24	32	40	100
4	C 4	Selliampara, Idukki	January, 2017	40.34 (39.42)^c	18	29	37	93
5	C 5	Devikulam, Idukki	January, 2017	45.07 (42.17)^b	18	29	37	93
6	C 6	Muthuvankudy, Idukki	January, 2017	57.14 (49.1)^a	18	29	37	93
7	C 7	Chithirapuram, Idukki	January, 2017	38.32 (38.24)^c	18	29	37	93

CD (0.05)	2.154				
SE	0.89				

*Mean of ten replications
PDI: Per cent Disease Index
Values in parenthesis are arcsine transformed values

Symptomatology

The disease specimens were collected during the survey and the symptomatology of the disease was studied. As a result of infection by the pathogen, brown to black lesions surrounded by yellow halo appeared initially on the lower leaves of cabbage. Later on these lesions enlarged in size and developed concentric zonations. As the disease progressed the number of lesions increased, and these lesions coalesced together forming patches which leads to blighting of the leaf. Under severe infestation water soaked lesions appeared on the cabbage head, they enlarged in size and spreads to entire head. During the final stage, the entire head exhibited rotting symptom and later on dried resulting in severe yield loss as the economic part of the cabbage is head (Table 2).

Ansari (1988) [1], Dillard *et al.* (1997) [4], Kucharek (2000) [6] and Doullah *et al.* (2006) [5] also stated that dark spots appear on lower leaves of cabbage due to infection by *A. brassicicola* and dark spots on leaves ranged from one to four cm in diameter and developed concentric rings within the spots. These spots coalesced and lead to defoliation and caused significant yield loss in cabbage.

Table 2: Stages of symptom development

Sl. No.	Stage	Symptom
1	Lesions developed on the leaf with an yellow halo	
2	Lesions enlarged and developed concentric rings	
3	Lesions blend together and formed patches	
4	Lesions developed, enlarged and coalesced together on head	
5	Entire head exhibited rotting symptom and dried	

Isolation, Purification and Pathogenicity testing

The pathogen causing *Alternaria* leaf spot of cabbage was isolated from the diseased samples collected during the survey. Seven fungal isolates were obtained from the diseased samples and were purified by single spore isolation method. Then these purified cultures were subcultured periodically on the PDA slants to continue further studies.

The pathogenicity was tested for seven isolates of *A. brassicicola* obtained during the survey were assessed by artificial inoculation on healthy plants. The lesion size and days taken for complete drying of the leaf were observed to find the most virulent isolate of the pathogen. The seven isolates of the pathogen were inoculated on the potted plants and the lesion size were noted upto eight days after inoculation (Table 3).

Table 3: Virulence testing of *A. brassicicola* isolates

Isolate	Lesion size (cm) *						
	2 DAI	3 DAI	4 DAI	5DAI	6DAI	7DAI	8DAI
C 1	-	0.5^{e}	0.9^{d}	1.3^{d}	1.8^{f}	2.4^{f}	3.1^{f}
C 2	0.7^{b}	1.4^{b}	2b	2.7^{b}	4^{b}	5.2^{b}	6.3^{b}
C 3	-	0.6^{e}	1.0^{d}	1.5^{d}	2.2^{e}	2.9^{e}	3.7^{e}
C 4	0.5^{c}	1.0^{d}	1.5^{c}	2.1^{c}	3^{d}	3.9^{d}	5.2^{d}
C 5	0.7^{b}	1.3^{c}	1.9^{b}	2.6^{b}	3.6^{c}	4.7^{c}	5.8^{c}
C 6	1.2^{a}	1.9^{a}	2.8^{a}	4.1^{a}	5.2^{a}	6.8^{a}	8.3^{a}
C 7	0.4^{c}	0.9^{d}	1.4^{c}	1.9^{c}	2.8^{d}	3.7^{d}	5.0^{d}
CD (0.05 level)	0.18	0.17	0.23	0.23	0.31	0.3	0.36

*Mean of three replications
DAI: Days after inoculation

Cultural characters of *Alternaria* leaf spot isolates

Cultural characters of the seven isolates were studied and observed that they differ in average growth rate, growth pattern and colony colour (Table 4). Maximum average growth rate was recorded in C6 isolate (0.64 cm/day) followed by C4 isolate (0.58 cm/day). The average growth rate was similar in C3 and C5 isolates (0.56 cm/day) followed by C7, C2 and C1 isolate which was 0.51, 0.50 and 0.46 cm/day. The days taken by the C6 isolate to cover the entire petriplate (9 cm) was 14 days. But the isolate C3, C4 and C5 take 16 days and C2 and C7 took 18 days to attain full growth in the petriplate. However the isolate C1 attained full growth in the petriplate only after 19 days. The colony colour of these isolates were initially white but later turns to brown in C1, C3, C4, C6 and C7 isolates whereas it turned to light brown in C2 isolate and to grey brown in C5 isolate (Table 4, Plate 1).

Colony characters of *A. brassicicola* were described as olive to grey brown in colour and showed velvety growth. The mycelium was septate, branched and produced conidiophores (Pattanamahakul and Strange, 1999) [10]. Rahimloo and Ghosta, (2015) [12] stated that the colony colour of 38 isolates of *A. brassicicola* were grey to brown and average growth rate was 0.78 cm /day.

Table 4: Cultural characters of *Alternaria* leaf spot isolates

Isolate	Growth in petriplate (cm) *	Average growth rate (cm/day) *	Growth pattern	Colony colour	DTCP
C 1	7.9	0.46	Dense	White turn to brown	19
C 2	8.1	0.50	Sparse	White turn to light brown	18
C 3	8.5	0.56	Sparse	White turn to brown	16
C 4	8.7	0.58	Dense	White turn to brown	16
C 5	8.5	0.56	Dense	White turn to grey brown	16
C 6	9	0.64	Dense	White turn to brown	14
C 7	8.2	0.51	Dense	White turn to brown	18
CD	0.15	0.016			
SE	0.06				

*Mean of three replications
DTCP: Days taken to cover 9 cm in petridish

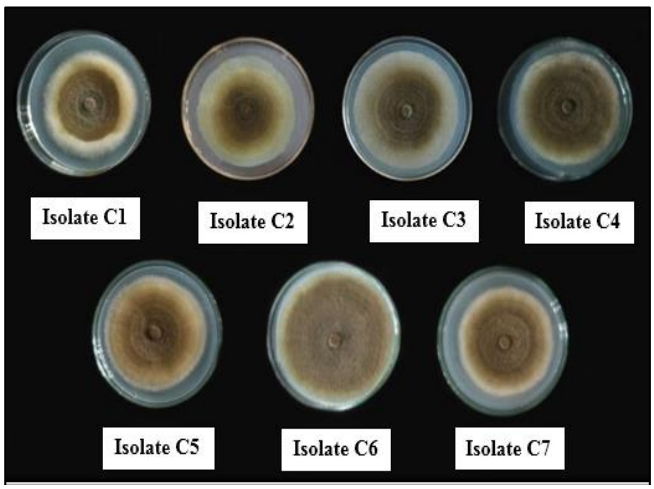


Plate 1: Growth of *Alternaria* spp. isolates obtained from different locations

Morphological characters of *Alternaria* leaf spot isolates

Morphological characters of these isolates were studied by observing the temporary slides prepared from the pure culture of the fungus as well as by slide culture technique and the observations regarding the morphological characters of all the isolates were mentioned in the Table 5 and plate 2.

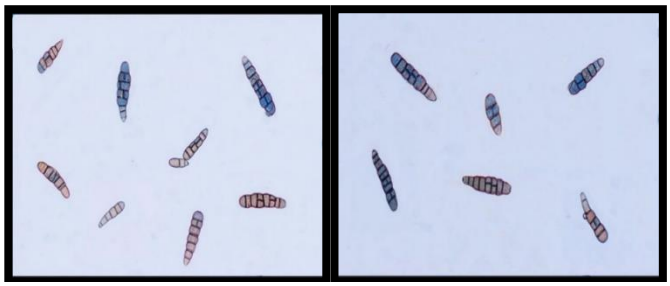
The hyphae of *Alternaria* spp. isolates were septate, branched and initially transparent or hyaline and later turn to light brown colour. Conidiophores developed from the hyphae were septate, upright or curved, olive grey to brown in colour and produced conidia at the apical portion. Conidia were muriform type with both longitudinal (0-3) and transverse septa (1-8) and the average conidial size ranges from 8-70 µm in length and 8-19 µm in width. These conidia were obpyriform in shape without beak, olive grey to brown in colour and produced in chains. Based on the morphological and colony characteristics all these seven isolates were tentatively identified as *A. brassicicola* (Wiltshire).

Pattanamahakul and Strange (1999) [10] also observed similar morphological characters as seen in the present study in *Alternaria* leaf spot causing pathogen, *A. brassicicola*. Meena *et al.* (2010) [8] reported that the mycelium of *A. brassicicola* were septate, branched and conidiophores were olive grey in colour. The conidia were beakless and number of transverse and longitudinal septa were 5-8 and 0-4 in number. Chauhan *et al*. (2009) [2] reported that conidiophores were septate (0-4) and olivaceous in colour. Rop *et al*. (2009) [15] reported that the conidia of *A. brassicicola* developed in chains from conidiophores and the length of young conidia was smaller than the initially developed conidia. Longitudinal (7) and transverse septa (1-11) were present in conidia with shallow constrictions at the septation point. The average diameter of cylindrical beakless conidia was 10-130 µm × 6-20 µm.

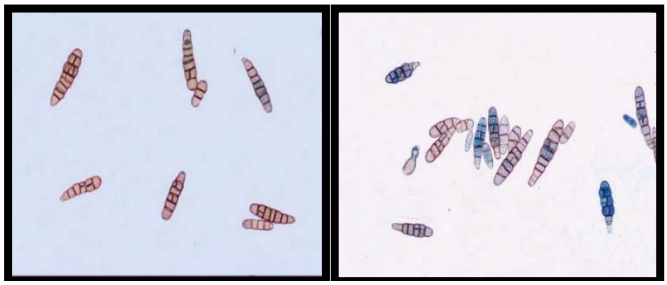
Table 5: Morphological characters of *Alternaria* leaf spot isolates

Isolate	Conidiophore	Conidia size		Conidia septation		Mycelial width (µm) *
		Length (µm)	Breadth (µm)	Transverse septa (no.)	Longitud-inal septa (no.)	
C 1	Olive grey to brown colour, 0-4 septate	10 - 58	6-17	1 – 9	0 – 3	8.31
C 2	Olive grey to brown colour, 0-4 septate	9- 64	8-17	1 – 9	0 – 3	8.26
C 3	Olive grey to brown colour, 0-4 septate	8- 66	7-17	1 – 9	0 – 3	8.37
C 4	Olive grey to brown colour, 0-4 septate	11- 70	7- 19	1 – 9	0 – 3	8.41
C 5	Olive grey to brown colour, 0-4 septate	10- 70	7- 19	1 – 9	0 – 3	8.28
C 6	Olive grey to brown colour, 0-4 septate	8- 70	8- 19	1 – 9	0 – 3	8.36
C 7	Olive grey to brown colour, 0-4 septate	9- 66	7- 17	1 – 9	0 – 3	8.4

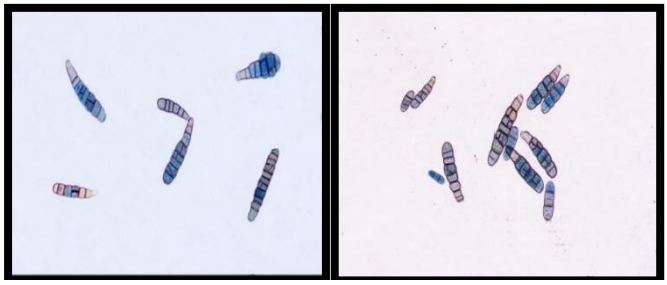
*Mean of 50 spores



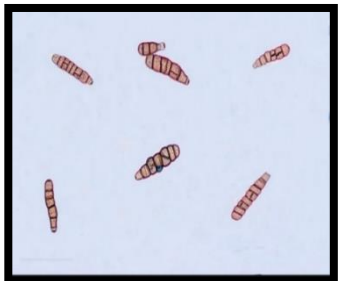
Isolate C1 Isolate C2



Isolate C3 Isolate C4



Isolate C5 Isolate C6



Isolate C1

Plate 2: Conidia of *Alternaria* leaf spot isolates

References

1. Ansari NA. Identity and cultural characters of the pathogen causing *Alternaria* blight of rapeseed and mustard. J Oilseeds Res. 1988; 5(2):80-88.
2. Chauhan JS, Badoni A, Singh NI, Ali S. Effect of *Alternaria* on some members of family brassicaceae of garhwal Himalayas. N. Y. Sci. J. 2009; 2(6):80-85.
3. Dhingra OD, Sinclair JB. Basic Plant pathology methods. CBC publications and distributors, New Delhi, 1985; 335.
4. Dillard HR, Cobb AC, Lamboy JS. Transmission of *Alternaria brassicicola* to cabbage by flea beetles (*Phyllotreta cruciferae*). Plant Dis. 1997; 82:153-157.
5. Doullah MAU, Meah MB, Okazaki K. Development of an effective screening method for partial resistance to *Alternaria brassicicola* (dark leaf spot) in *Brassica rapa*. Eur. J Plant Pathol. 2006; 116:33-43.
6. Kucharek T. *Alternaria* diseases of crucifers. Plant Pathol. 2000; 7(3):34-38
7. McKinney HH. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. J Agric. Res. 1923; 26:195-217.
8. Meena PD, Aswathi RP, Chattopadhyay C, Kolte SJ, Kumar A. *Alternaria* blight: A chronic disease in rapeseed and mustard. J. Oilseed Brassica. 2010; 1(11):1-11.
9. Michereff SJ, Naronha MA, Filha MSX, Camara MPS, Reis A. Survey and prevalence of species causing *Alternaria* leaf spots in brassica species in Pernambuco. Hotric. Bras. 2012; 30(2):1-6.
10. Pattanamahakul P, Strange RN. Identification and toxicity of *Alternaria brassicicola*, the causal agent of dark leaf spot disease of brassica species grown in Thailand. Plant Pathol. 1999; 48:749-755.
11. Peruch LAM, Michereff SJ, Araujo IB. Survey of the intensity of *Alternaria* black spot and black rot on brassica species under organic farming systems in Pernambuco and santa Catarina states, brazil. Hortic. Bras. 2006; 24(4):1-7.
12. Rahimloo T, Ghosta Y. The occurrence of *Alternaria* species on cabbage in Iran. Zemdirbyste. Agric. 2015; 102(3):343-350.

13. Reis A, Boiteux LS. *Alternaria* species infecting brassicaceae in the Brazilian neotropics: geographical distribution, host range and specificity. J Plant Pathol. 2010; 92(3):661-668.
14. Riddle RW. Permanent stained mycological preparation obtained by slide culture. Mycologia. 1950; 42:265-270.
15. Rop NK, Kiprop EK, Ochuodho JO. *Alternaria* species causing black spot disease of brassicas in Kenya. Afr. Crop Sci. Conf. Proc. 2009; 9:635-640.
16. Sangeetha CG, Siddaramaiah, AL. Epidemiological studies of white rust, downy mildew and *Alternaria* blight of Indian mustard. Afr. J Agric. Res. 2007; 2(7):305-308.